preventing the development of resistance in pest insects.

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Characterisation of the relationship between binding sites for imidacloprid and other nicotinic ligands in insects

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Abstract: Radioligand binding studies using the neurotoxins α-bungarotoxin, epibatidine, imidacloprid (IMI) and methyllycaconitine reveal heterogeneity at the level of the nicotinic acetylcholine receptor (nAChR) in membranes from the peach potato aphid *Myzus persicae* (Sulzer) and further suggest the presence of more than one ligand binding site per nAChR. These sites are able to interact allosterically with each other. Of particular interest, [^{3}H]IMI has over an order of magnitude higher affinity in membranes of hemipteran pest species than in non-hemipteran insects, which may help explain why IMI is particularly effective for the control of sucking pests.

Keywords: *Myzus persicae*; nicotinic acetylcholine receptor; imidacloprid; methyllycaconitine; epibatidine; radioligand binding

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Table 1. Comparison of [^{3}H]IMI saturable binding in a panel of insects. Data for *Myzus persicae* is the mean (±SEM) of n=5, and n=1 for other insects

	High affinity		*Low affinity*		
Insect[a]	K_d (nM)	B_{max} (fmol mg^{-1})	K_d (nM)	B_{max} (fmol mg^{-1})	*Hill value* (n_H)
M persicae	0.14 (±0.01)	284 (±16)	12.58 (±1.83)	883 (±142)	0.61 (±0.02)
B tabaci	0.10	19	2.1	112	0.50
N cincticeps	<0.01	33	1.23	179	0.67
*H virescens**	1.51	134			0.92
D melanogaster	1.42	126			1.04
*P americana**	3.14	2140			1.01
C felis	4.82	369			0.95

* Heads only.

1 INTRODUCTION

The naturally occurring neurotoxins α-bungarotoxin (α-BgTx), epibatidine (EPI) and methyllycaconitine (MLA), all known to interact specifically with vertebrate nicotinic acetylcholine receptors (nAChRs), were used to investigate nAChR of insects, principally the aphid *Myzus persicae* (Sulzer). The synthetic neonicotinoid insecticide imidacloprid (IMI), which is particularly effective against sucking insect pests such as aphids, was also studied.[1]

2 EXPERIMENTAL METHODS

[^{3}H]EPI (55Ci mmol^{-1}), [^{3}H]IMI (30Ci mmol^{-1}), unlabelled IMI and [^{3}H]α-BgTx (60Ci mmol^{-1}) were supplied by Zeneca Agrochemicals. Na^{125}I, obtained from Amersham International (UK), was used to iodinate α-BgTx to a specific activity of 750 Ci mmol^{-1}. [^{3}H]MLA (~50 Ci mmol^{-1}) was synthesised by Tocris Cookson Ltd, Bristol, England. Other unlabelled drugs were obtained from Sigma-Aldrich Ltd (Poole, Dorset, UK).

Insect membranes were prepared as described elsewhere for whole peach potato aphid *M persicae*, cotton whitefly *Bemisia tabaci* (Gennadius), green leaf hopper *Nephotettix cincticeps* (Uhler), cat flea *Ctenocephalides felis* (Bouche), and fruitfly *Drosophila melanogaster* (Meigen), and heads of the tobacco budworm *Heliothis virescens* (F) and the cockroach *Periplaneta americana* (L).[2] Saturation, displacement and dissociation experiments employed either filtration or centrifugation to separate bound from free ligand using a cell harvester. Radioactivity was determined with scintillation spectrometry.[2] Isotopic dissociation studies employed either 1 μM α-BgTx, EPI, IMI or MLA to initiate dissociation from *M persicae* membranes after an association period of 3 h with [^{3}H]α-BgTx. MLA was used at 1 μM to determine non-specific binding for all experiments.

3 RESULTS

Saturable binding in *M persicae* membranes demonstrated that [^{125}I]α-BgTx (K_d = 1.2 & 34 nM, B_{max} = 167 & 640 fmol mg^{-1}), [^{3}H]EPI (K_d = 0.9 & 18 nM, B_{max} = 344 & 904 fmol mg^{-1}) and [^{3}H]IMI (K_d = 0.14 & 13 nM, B_{max} = 284 & 883 fmol mg^{-1}) each labelled two sites. In contrast, [^{3}H]MLA binding was resolvable as only a single binding site with a K_d of 0.95 nM and a B_{max} of 1290 fmol mg^{-1}. Similar studies using membranes of two hemipteran insects, *B tabaci* and *N cincticeps*, also revealed the presence of high- and low-affinity [^{3}H]IMI binding components. By contrast, studies in non-hemipteran insects could only resolve a single class of [^{3}H]IMI binding site. Of particular interest, the high affinity site labelled by [^{3}H]IMI in all of these hemipteran insects was at least an order of magnitude higher in affinity than was the single site in non-hemipteran insects (Table 1).

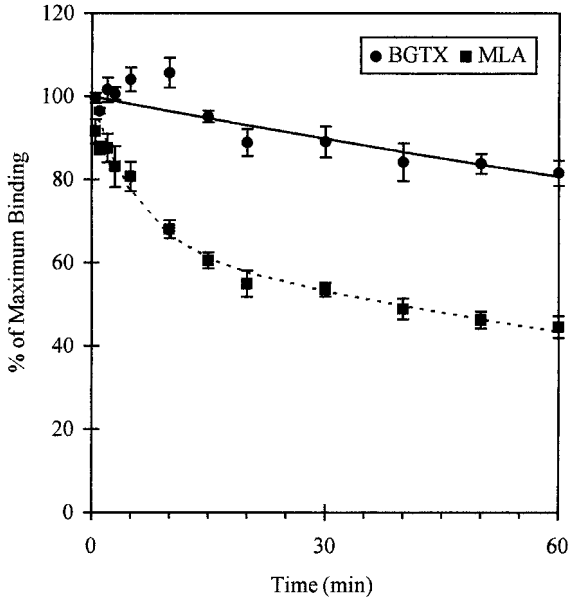

Figure 1. Isotopic dissociation of [^{3}H]α-BgTx in *M persicae* membranes initiated by either 1 μM α-BgTx or MLA. Data shown are the mean of three experiments (±SEM).

The rate of [^{3}H]α-BgTx isotopic dissociation was slow and monophasic from its high affinity binding site in *M persicae* membranes when initiated by either 1 μM

α-BgTx, EPI or IMI. In contrast the dissociation rate was markedly increased when initiated by MLA compared to α-BgTx, EPI or IMI (Fig 1).

Displacement of [^{125}I]α-BgTx, [^{3}H]EPI and [^{3}H]IMI by MLA was with high potency with an IC_{50} value close to the K_d value of each of these labelled ligands. Displacement of [^{3}H]MLA by α-BgTx, EPI and IMI resulted in low potency, but was characterised by shallow displacement curves.

4 DISCUSSION AND CONCLUSIONS

Saturation studies in *M persicae* membranes demonstrate that [^{125}I]α-BgTx, [^{3}H]EPI, and [^{3}H]IMI all label two binding components of differing affinities in a ratio of 1:3. In each case the sum of the high and low affinity B_{max} values approximated the B_{max} value of the single site labelled by [^{3}H]MLA. [^{3}H]MLA is a novel radioligand which is highly selective in vertebrate nAChRs,[3] but which appears to be unable to distinguish between a heterogeneous population of nicotinic ligand binding sites in insects.

The increase in dissociation rate of [^{3}H]α-BgTx using MLA instead of either α-BgTx, EPI or IMI is consistent with an allosteric interaction by MLA at a binding site other than that of the high affinity [^{3}H]α-BgTx binding site.

Displacement studies have shown that MLA is a potent ligand at sites labelled with high affinity by [^{125}I]α-BgTx, [^{3}H]EPI, and [^{3}H]IMI. The shallow displacement curves obtained when [^{3}H]MLA is displaced by α-BgTx, EPI, and IMI (data not shown) are likely to be due to displacement of [^{3}H]MLA from at least two distinct binding sites, for which the displacing ligands have different affinities, but which are indistinguishable by [^{3}H]MLA.

Interestingly, the high affinity binding of [^{3}H]IMI in hemipteran membranes compared to that in non-hemipteran insects may help explain why IMI is particularly useful for the control of sucking pests.

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Nicotinic acetylcholine receptor chimeras of rat α7 and *Drosophila* SAD reveal species-specific agonist binding regions

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Abstract: Species-specific agonist binding regions of nicotinic acetylcholine receptors (nAChR) were examined. Imidacloprid and physostigmine (Phy) selectively activated insect nAChR composed of *Drosophila* second alpha-like subunit (SAD) and chick β2, in contrast to rat α7 nAChR. The Phy-activated currents were α-bungarotoxin (α-BGT) sensitive, suggesting activation at the agonist binding loop C. Several SAD-α7 chimeras were constructed, by switching agonist binding regions, and expressed in oocytes. Though none of the chimeras was activated by a range of nicotinic agonists, [^{125}I]α-BGT binding revealed homomeric assembly of all chimeric cDNAs. Phy differentially displaced [^{125}I]α-BGT from the nAChR chimeras, suggesting that the β subunit is not involved in Phy binding, and that Phy targets the insect agonist binding loop C.

Keywords: nicotinic receptor; agonist binding; chimera; physostigmine; imidacloprid; acetylcholine receptor; neonicotinoid

1 INTRODUCTION

Multiple nicotinic acetylcholine receptor (nAChR) subtypes exist, both within and between species. Insects express only neuronal nAChR, while, in vertebrates, endplate and neuronal nAChR types are distinguished. A range of different insect and vertebrate α subunits exist, which may be combined with various β subunits to form functional nAChR. Three loops in the α subunits are thought to be involved in acetylcholine (ACh) binding,[1] ie loop A (residues 86–93), loop B (residues 148–151), and loop C (residues 190–198). The latter includes the two adjacent cysteines characteristic of α subunits. Distinct nAChR subtypes are distinguished by their physiological and pharmacological properties. In addition, differential sensitivities to chemical compounds may occur, suggesting a role in species-selective toxicity. The nitroguanidine insecticide imidacloprid selectively activates insect nAChR by binding to the agonist

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